Environmental Factors Contributing to the Development of Autism Spectrum Disorder – a Large Database Retrospective Study

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Abstract

Autism Spectrum Disorder (ASD) is a complex, serious, biologically based disorder of brain development. It affects the brain, immune system and the gut of children. The cause of ASD is not well established and the subject of intense inquiry. Starting in 1988 the rate of children developing ASD increased dramatically. The US prevalence rate has now skyrocketed to more than one percent of all US children in 2009. For male children the rates are even more staggering – one in 63 or 2.6% of all male children now being born in America. There is no consensus on the cause of autism.

Over the last several years there has been renewed interest and research into the interaction between vulnerable genes and environmental triggers with a growing sense that low-dose, multiple toxic and infectious exposures may be a key contributing factor to ASD onset. A recent EPA study concludes that environmental factors have contributed to the increase rate of autism.

Results from a retrospective data analysis of a very large ASD clinical database shows confirming information that indeed children with ASD have elevated levels of several toxic chemicals that have been documented to be neurotoxic and that these same children have genetic variations that interfere with the proper detoxification of these chemicals, suggesting a causative explanation, With over 2,000 patients in the database, this may be one of the largest studies to show that environmental factors and associated genetic components may be contributing to the causation of ASD.

Introduction

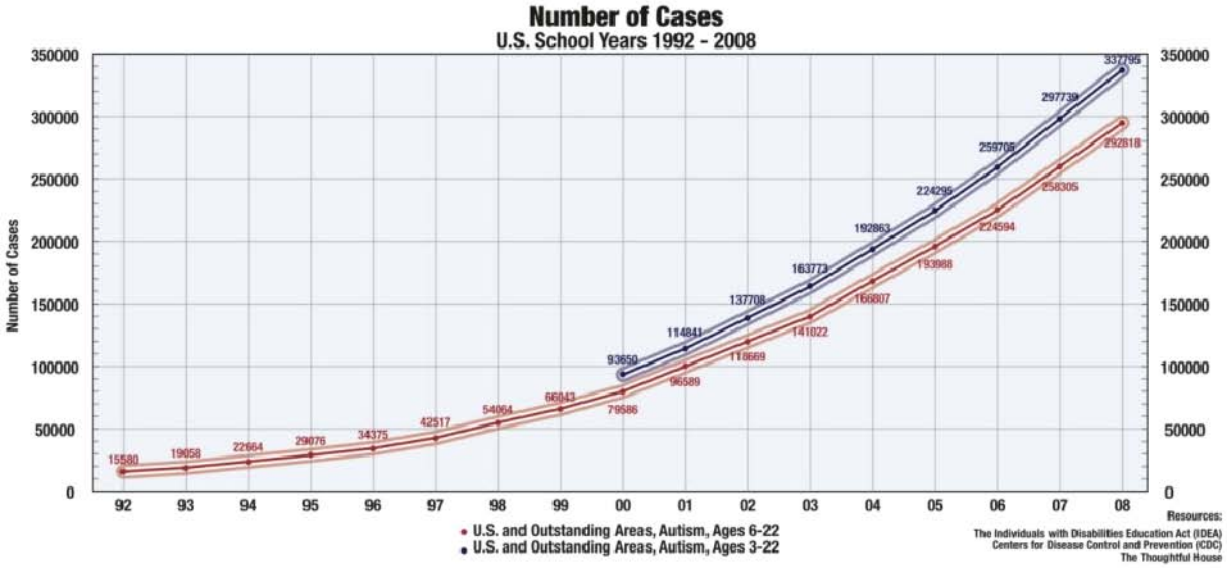
Autism is a complex, serious, biologically based disorder of brain development first described in 1943 by Kanner.[i] Social deficits, abnormalities in communication, repetitive behaviors, and cognitive inflexibility are the characteristic features. There is no specific biochemical indicator or distinct neuro-anatomical abnormality that defines autism, and the diagnosis is based on clinical and behavioral assessment.[ii]
Cases of autism vary from mild to profound and in the relative prominence of particular features and comorbidities. Approximately 50% of autistic children have intellectual disability, some have abnormally increased brain size, one-third have had at least two epileptic seizures by late adolescence, and about half have severely impaired speech.[iii] Yet some children with autism, notably those with Asperger's syndrome, have highly developed intellectual skills, sometimes in specific areas such as mathematics.

Because of this heterogeneity, the term 'autism spectrum disorder' (ASD) has come into use. ASD encompasses autistic disorder, Asperger's syndrome and pervasive developmental. [iv]

The causation of autism is the subject of intense inquiry. Genetic factors are clearly important. Gene mutations, gene deletions, copy number variants (CNVs) and other genetic anomalies are all persuasively linked to autism. But none accounts for more than a relatively small fraction of cases. The hypothesis therefore arises that early environmental exposures may also contribute to causation, perhaps acting in concert with genetic susceptibilities single nucleotide polymorphisms (SNPs). It may further be hypothesized that variation in the interplay between different environmental exposures and inherited vulnerabilities may account for the observed heterogeneity in the autism phenotype. Etiologic hypotheses include: genetic predisposition to Autism including impaired methylation capacity with resultant inability to clear heavy metals, increased vulnerability to oxidative stress, and impaired neurological adaptability function; environmental exposures including mercury preservatives in vaccines, trans-generational accumulation of heavy metals and biological conditions. Autism is best redefined as an environmental disease with genetic susceptibilities at it's core.

The Problem

The incidence of autism in the United States has reached epidemic proportions increasing almost sixty-fold since the 1970s with the most dramatic increase during the last 10 years. [v] [vi] The condition most commonly presents in early childhood and occurs in males four times more frequently than in females. ASD is now affecting approximately 2.6% of all male children (1 in 63) and 1% of all children (1 in 100). [vii] [viii] People with autism in California born between 1988 and 1997 will incur a mean lifetime care cost between $2.7 billion and $4.0 billion, and these costs are likely to grow in coming years. Factor these costs from this one state across the entire country and the costs become staggering and potentially financially catastrophic. The US spends an estimated $35 to $60 billion annually on autism-related costs alone. [ix] The bill for the tide of autistic children entering adulthood over the next 15 years, an estimated $27 billion annually in current, non-inflation-adjusted dollars by the end of that period. The number of autistic children expected to need extensive adult services by 2023 -- more than 380,000 people – which is roughly equal to the population of Minneapolis. [x] If a town were created to house this group of people and their caregivers -- for you can't separate the two -- it would exceed the population of all but six U.S. cities. If they formed a state, it would have four electoral votes.



The Cause of ASD

The physiological abnormalities that occur in autism appear in most cases to be due to a combination of genetic propensity and environmental insult. Since it is impossible to have a "genetic epidemic", one must examine possible early environmental insults (exacerbation or triggering by toxins, infectious agents or other stressors) for clues to explain the increase in autism cases.
While autism has traditionally been modeled as a brain disorder emerging findings and hypothesis support a broader model of the condition as both genetically influenced and systemic associated with gastrointestinal, neuroinflammatory and immune disorders.

The role of environmental factors — particularly toxic chemical exposures — in the onset, trajectory, and overall incidence of a number of diseases and disabilities is often overlooked. While adverse health outcomes are the result of a complex interplay of multiple factors, including heredity, gene-environment interaction, nutrition, and socioeconomic status, both laboratory and human studies indicate that toxic chemical exposures may play a role. Scientific evidence implicates environmental exposures as discernable contributors to adverse health outcomes, such as cancer, neurodegenerative diseases, reproductive health problems, and learning and developmental disabilities. [xi]

"*Recent increases in chronic diseases like diabetes, childhood asthma, obesity or **autism** cannot be due to major shifts in the human gene pool as those changes take much more time to occur. They **must** be due to changes in the environment, including diet and physical activity, which may produce disease in genetically predisposed persons*" Francis Collins, MD, NIH Director

It seems prudent to assume that at least some portion of this increase in incidence of autism is real and results from environmental factors interacting with susceptible populations. As such exposure is potentially preventable, identification of relevant candidate environmental factors should be a research priority. EPA Report, Michael McDonald MD

Current understanding of the exquisite vulnerability of the developing brain to toxic exposures in the environment leads to support that there

is an environmental contribution to the causation of autism. Some of the current research areas for ASD environmental triggers include: Air Pollution, Endocrine Disruptors, Pesticides, Mercury in fish and other products, Retroviruses, Vaccines and underlying disorders, Vaccines and Immune Stress and Vaccines and Viral Particles.

Treatment of ASD

Current treatments for autism can divided into behavioral, nutritional, medical and bio-medical approaches, although no clear golden standard approach exists. Behavioral interventions usually include activities designed to encourage social interaction, communication, awareness of self, and increase attention. Nutritional interventions aim to restrict allergy-associated dietary components, as well as to supplement minerals or vitamins that may be lacking, address gastrointestinal dysbiosis, immune system support and measures to reduce inflammation. Bio-medical interventions attempt to modify the expression of genetic predispositions. Medical interventions usually treat specific activities associated with autism through drug therapy.

Genetics

Virtually all human diseases result from the interaction of genetic susceptibility and modifiable environmental factors, broadly defined to include infections, chemical, physical, nutritional and behavioral influences. Genetics is about vulnerability.

Slight variations in genetic makeup called Single Nucleotide Polymorphism (SNPs) are associated with almost all disease. Genetic variations themselves do not cause disease but rather influence a person's susceptibility to specific environmental factors that increase disease risk.

Personalized Medicine

Personalized medicine is the application of genomic and molecular data to better target the delivery of health care, facilitate the discovery and clinical testing of new products, and help determine a person's predisposition to a particular disease or condition and their responsiveness to specific treatment options. The overarching concept is that information about a patient's protein, gene or metabolic profile could be used to tailor medical care to his or her needs.

CARE Clinics

CARE Clinics was founded to provide cutting edge medical care for people with ASD. The clinic, located in Austin Texas, treated thousands of children with autism utilizing a personalized medicine biomedical approach. Utilizing a battery of diagnostic testing the clinic attempted to understand the etiology of each patient's condition and develop a personalized treatment rationale.

The clinics extensive digital database provides a unique and valuable opportunity to study the connections between many of the proposed causes of ASD and their relationship to effective treatment.

The database was analyzed comparing ASD patients to either non-ASD clinic patients (controls) and/or laboratory/published historical reference ranges. Due to the large number of subjects the results had a high degree of statistical power and significance. All reported results were statistically significant with a two-tailed p of less then 0.0001 with a 95% confidence interval. Where appropriate a nonparametric test rejected the null hypothesis. It is the author's belief that this is the first time such significant results have been reported with the large number of subjects. All laboratory tests were performed at CLIA certified laboratories. The control group consisted of non ASD patients, often relatives of the ASD children.

The Environment and Toxins

There is a growing sense that our heavily industrialized, chemical-soaked environment—and the way it acts on vulnerable genes in some individuals—may be a major culprit in the etiology of ASD. In December 2006, Harvard researchers announced in The Lancet that industrial chemicals may be impairing the brain development of children around the entire world. The researchers found that 202 industrial chemicals have the capacity to damage the human brain, and they conclude that chemical pollution may have harmed the brains of millions of children worldwide. The authors conclude further that the toxic effects of industrial chemicals on children have generally been overlooked. The researchers say that neurodevelopmental disorders of possible environmental origin affect between 5% and 10% of babies born worldwide, leading to dyslexia, mental retardation, attention deficit/hyperactivity disorder, cerebral palsy, and autism.

At a November 2006 conference at the University of California at Davis's M.I.N.D. Institute, Dr. Issac Pessah gathered experts to discuss the clinical implications of environmental toxicology in autism. Says Dr. Pessah, "We discussed the enormous number of chemicals in our environment and how little we know about chronic, low-dose, multiple exposures and their effect on diseases like autism. Maybe the many autism cases we are now seeing are a new illness of the current generation."

Indirect evidence for an environmental contribution to autism comes from studies demonstrating the sensitivity of the developing brain to external exposures such as lead, ethyl alcohol and methyl mercury. But the most powerful proof-of-concept evidence derives from studies specifically linking autism to exposures in early pregnancy - thalidomide, misoprostol, and valproic acid; maternal rubella infection; and the organophosphate insecticide, chlorpyrifos. [xii]

Fetal and early childhood exposures to industrial chemicals in the environment can damage the developing brain and can lead to neuro-developmental disorders (NDDs)--autism, attention deficit disorder (ADHD), and mental retardation. [xiii]

Long and tragic experience that began with studies of lead and methylmercury has documented that toxic chemicals can damage the developing human brain to produce a spectrum of neurodevelopmental disorders ranging from overt toxicity at high levels of exposure down to subclinical dysfunction. [xiv] [xv] The vulnerability may be greatest during the first trimester of pregnancy. A growing list of chemicals is now implicated in causation of neurodevelopmental disabilities, including:

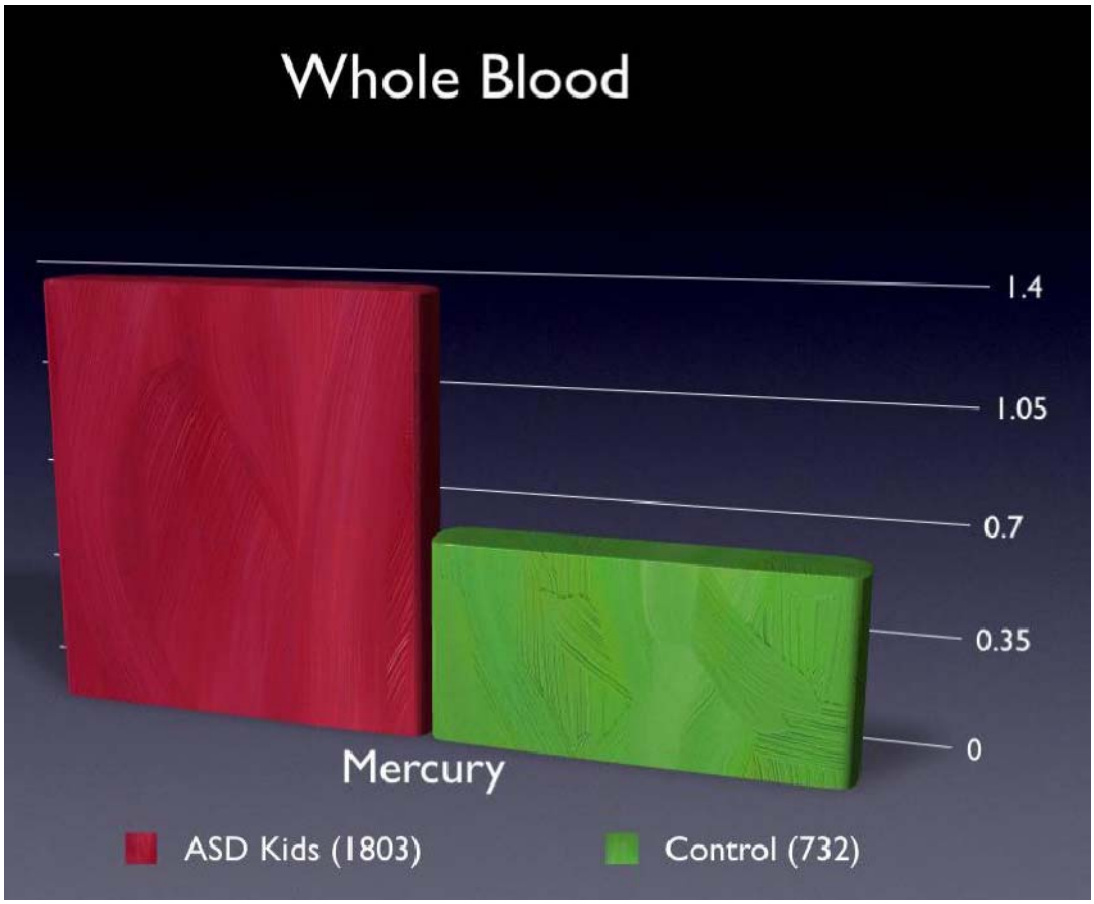
Lead, Methylmercury, Polychorlinated biphenyls, Arsenic, Manganese, Organophosphate insecticides, DDT and ethyl alcohol. [xvi]

CARE Clinics Data Analysis Results

A. Mercury - the amount of mercury was measured both in whole blood and after a provocative challenge in urine.

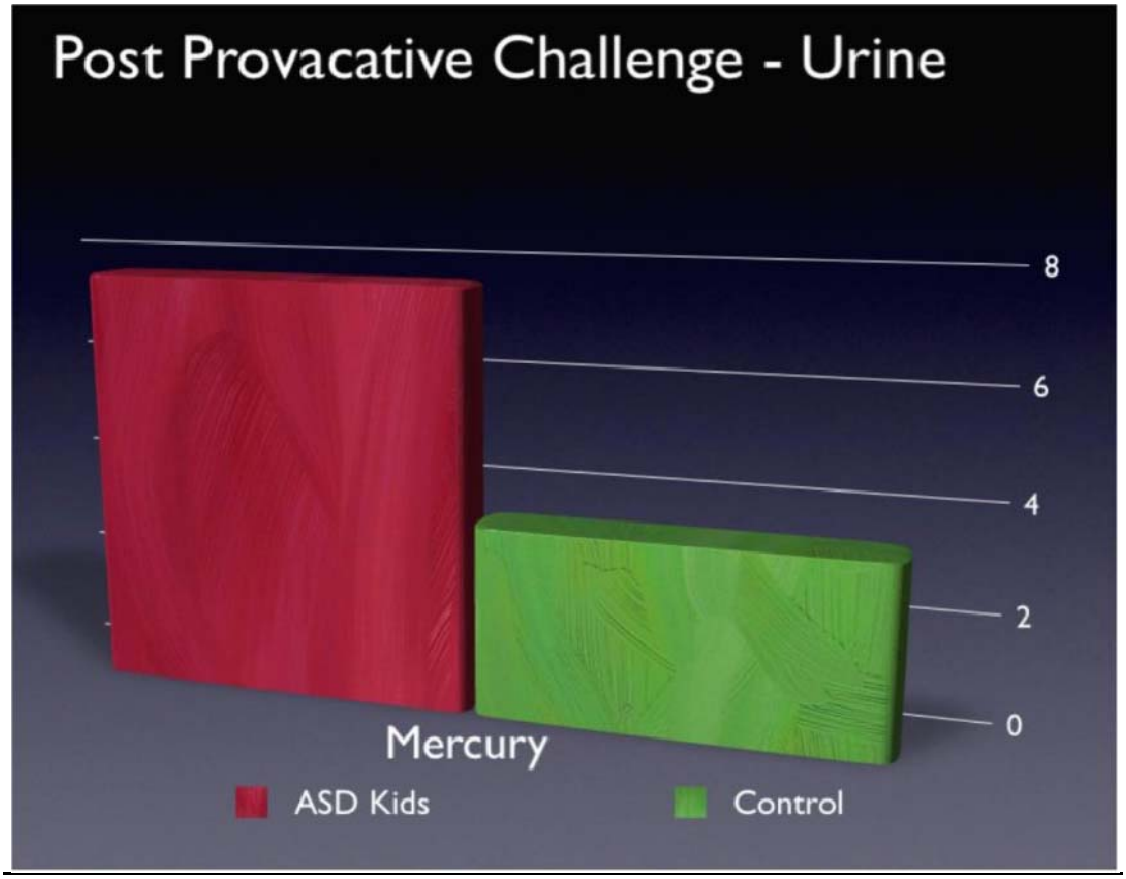
   a. Whole Blood Mercury levels were found to be elevated in ASD patients as compared to controls and to standard reference ranges. This is in agreement with previously published studies. [xvii]

      ASD Child Hg Whole Blood mean: 1.38 ug/g (n=1803)
      Control Hg Whole Blood mean: 0.73 ug/g (n=710)
      ref range nl <.1.0



b. Challenge
The amount of mercury was measured pre and post a provocative IV challenge. IV provocative testing of the urine is one the most accurate and reliable heavy metal testing methods available. [xviii]The challenge mixture included EDTA, DMPS and GSH. The 6 hour urine Post collection for ASD children found mercury levels to be highly elevated as compared to controls.

POST Challenge - Hg
Child ASD mean: 7.61 ug/g creatinine (nl is <5) (n=1905)
Adult mean: 4.45 ug/g (n=315)



Mercury

Recent studies hint that exposure to the toxic chemicals, such as methylmercury can cause harm at levels previously considered safe. [xix] A new analysis of the epidemiological evidence in the International Journal of Environment and Health, suggests that we should take a precautionary approach to this and similar compounds to protect unborn children from irreversible brain damage.

Heavy metal toxicity has emerged as a primary etiologic focus in ASD, with much emphasis on mercury exposure derivative of vaccines, dental amalgams and environmental load from ingestion of contaminated seafood. [xx] It is believed that the physiological effects of heavy metals are mediated through interference with protein synthesis and subsequent structure and function of enzymes. [xxi]

Philippe Grandjean of the Department of Environmental Health at Harvard School of Public Health, in Boston, and the University of Southern Denmark in Odense, explains that the causes of suboptimal and abnormal mental development are mostly unknown. However, severe exposure to pollutants during the development of the growing fetus can cause problems that become apparent as brain functions develop - and ultimately decline - in later life. Critically, much smaller doses of chemicals, such as the neurotoxic compound methylmercury, can harm the developing brain to a much greater extent than the adult brain. [xxii]

Methylmercury is a chemical compound formed in the environment from released mercury. Unfortunately, the methylmercury can be transported quickly around the body and may enter the brain. Serious problems will ensue if important developmental processes are blocked as there will be only one chance for the brain to develop.
Dr. Grandjean reports that neurodevelopmental disorders of possible environmental origin affect between 5% and 10% of babies born worldwide, leading to dyslexia, mental retardation, attention deficit/hyperactivity disorder, cerebral palsy, and autism.

Elevated body levels of heavy metals are reported in many ASD subjects [xxiii],[xxiv],[xxv] with excess of mercury in early baby teeth. Urinary porphyrin levels (a marker of heavy metal toxicity) were significantly elevated in children with autistic disorder. Removal of heavy metals by chelation

reduced urinary porphyrin levels pointing to, but not proving, a cause-effect relationship. A later report reiterated the porphyrin excess in ASD subjects. [xxvi]

Some researchers have described that Hg may have the potential to remain in the brain from several years to decades following exposure. [xxvii]The importance of persistent increased brain Hg levels stems from the fact that researchers have long recognized Hg is a neurodevelopmental poison This means that Hg exposure can severely disrupt the normal neurodevelopmental processes in the human brain. As a result, Hg may cause problems in normal neuronal cell migration and division, as well as inducing neuronal cell degeneration, and ultimately cell death. Based upon this knowledge, for example, Nelson from the National Institute for Occupational Safety and Health (NIOSH) of the US CDC reported that organic Hg was among the compounds known to induce behavior disorders such as autism. Subsequently, other researchers reported the specific biological effects of Hg exposure on neuronal development to be compatible with brain pathology observed in.

DeSoto and Hitlan postulated that if Hg does play any causal role in facilitating an ASD diagnosis, there would likely be at least some correlation between high Hg measured in the blood and the symptoms of autism, even if an individual's ability to metabolize mercury mediates the relationship between exposure and neural toxicity. [xxviii] This is because even if exposure is identical, those who remove Hg less effectively should still have higher levels in the blood. Subsequently, these researchers analyzed blood Hg levels in a cohort of children from China (ASDs and controls). These researchers concluded that a statistically significant relationship exists between total blood Hg levels and a diagnosis of an ASD.

A recent study [xxix] showed results in agreement with CARE Clinic results and indicated that subjects diagnosed with an ASD have, on average, significantly higher levels of Hg in their blood than controls. The neurotoxicity of Hg is well-established, and it is known that even small amounts of Hg can cause neurological injury similar to the brain pathology found in subjects diagnosed with an ASD. [xxx] In addition, recent research indicates subjects diagnosed with an ASD have a

decreased detoxification capacity for Hg. [xxxi] The weight of evidence provided by a variety of different studies offers a compelling argument for the hypothesis that Hg is a causal factor in the neuropathology reported in subjects diagnosed with an ASD.

Recent mercury research examples:

1. Porphyrinuria in Childhood Autistic Disorder: Implications for Environmental Toxicity
Toxicology and Applied Pharmacology, 2006. Robert Nataf, Corinne Skorupka, Lorene Amet
This study from France utilizes a new and sophisticated measurement for environmental toxicity by assessing porphyrin levels in autistic children. It provides clear and unequivocal evidence that children with autism spectrum disorders are more toxic than their neurotypical peers. The data clearly implicates environmental toxicity in childhood ASD.

2. A Case Control Study of Mercury Burden in Children with Autism Spectrum Disorder.
Journal of American Physicians and Surgeon, 2003. James Adams, PhD [Arizona State University].
This study shows, through active chelation with DMSA, that autistic children excrete significantly higher levels of mercury than their neurotypical peers, leading to the conclusion that autistic children bear a much higher load of mercury in their bodies and that chelation may be an effective treatment for removing the mercury.

3. A Case Series of Children with Apparent Mercury Toxic Encephalopathies Manifesting with Clinical Symptoms of Regressive Autistic Disorder
Journal of Toxicology and Environmental Health, 2007 David A. Geier, Mark R. Geier
This study reviewed the case histories and medical profiles of nine autistic children and concluded that eight of the nine children were mercury toxic and this toxicity manifested itself in a manner consistent with Autism Spectrum

Disorders. these previously normally developing children suffered mercury toxic encephalopathies that manifested with clinical symptoms consistent with regressive ASDs.

4. Attention-deficit hyperactivity disorder and blood mercury level: a case-control study in Chinese children *Neuropediatrics*, August 2006 P.R. Kong [Department of Pediatrics and Adolescent Medicine, The University of Hong Kong].
This study demonstrates that blood mercury levels are higher for children with ADHD. There was significant difference in blood mercury levels between cases and controls, which persists after adjustment for age, gender and parental occupational status. The geometric mean blood mercury level was also significantly higher in children with inattentive and combined subtypes of ADHD.

5. Higher levels of environmental mercury has been shown to produce higher rates of autism.
Environmental mercury release, special education rates, and autism disorder: an ecological study of Texas.

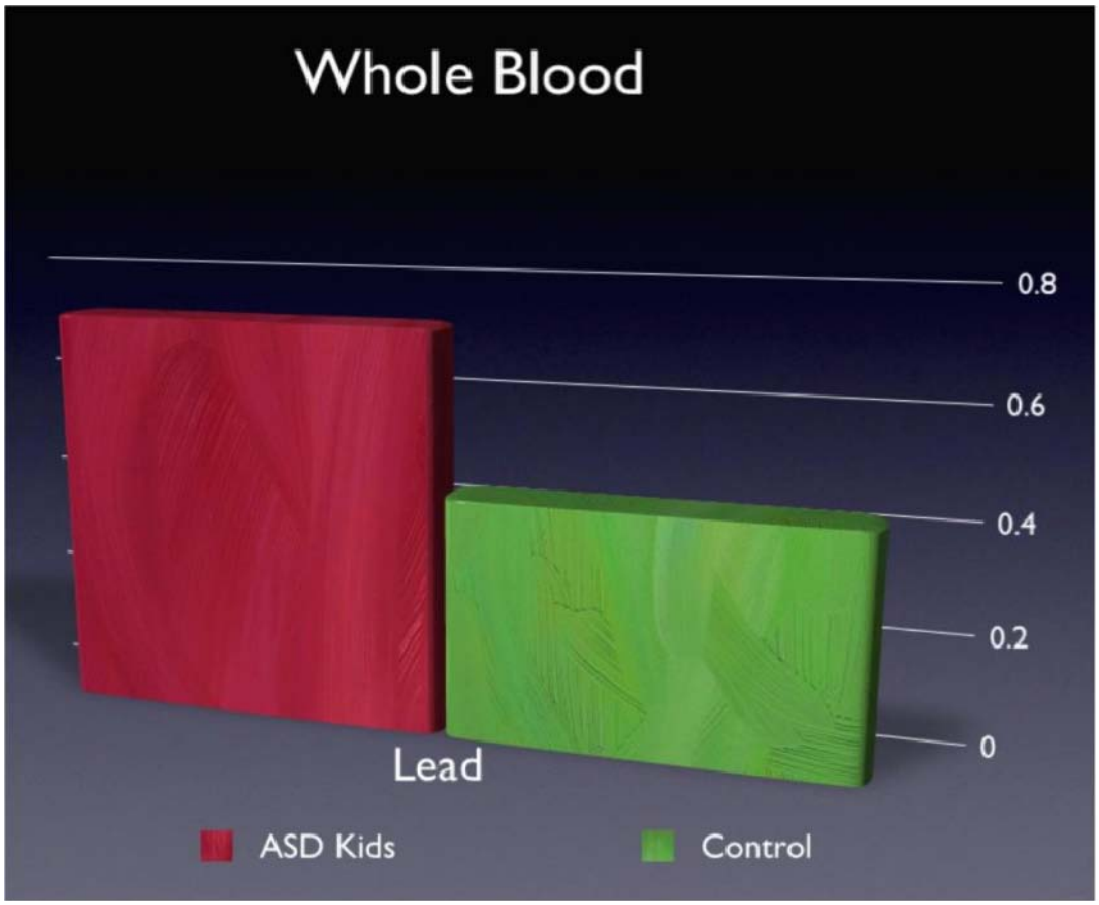
6. Health & Place, 2006 Raymond F. Palmer, University of Texas Health Science Center. This study demonstrated the correlation between environmental mercury and autism rates in Texas. *On average, for each 1,000 lb of environmentally released mercury, there was a 43% increase in the rate of special education services and a 61% increase in the rate of autism. The association between environmentally released mercury and special education rates were fully mediated by increased autism rates.*

B. Lead - the amount of lead was measured both in whole blood and after a provocative challenge in urine.

    a. Whole blood Lead levels were found to be elevated in ASD patients as compared to controls and to standard reference ranges. This is in agreement with previously

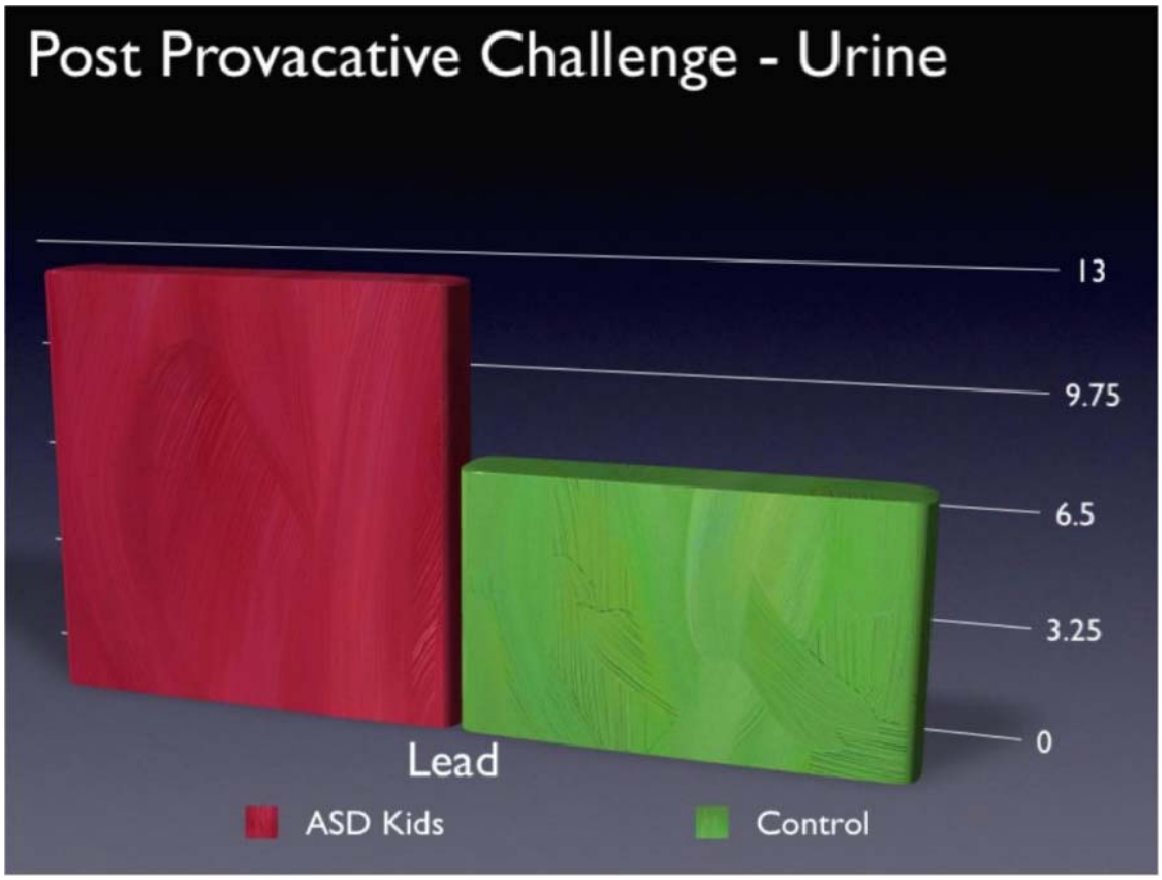
published studies. [xxxii]

ASD Child Pb Whole Blood mean: 0.72 ug/g (n=1803)
Control Pb Whole Blood mean: 0.43 ug/g (n=908)
ref range nl <.0.50 ug/g



b. The amount of lead was measured pre and post a provocative IV challenge. The 24 hour urine Post collection for ASD children found lead levels to be highly elevated as compared to controls.

POST Challenge - Pb
ASD Child mean: 12.58 ug/g creatinine (nl=<5) (n=2000)
Control mean: 8.64 ug/g (n=908)



Lead

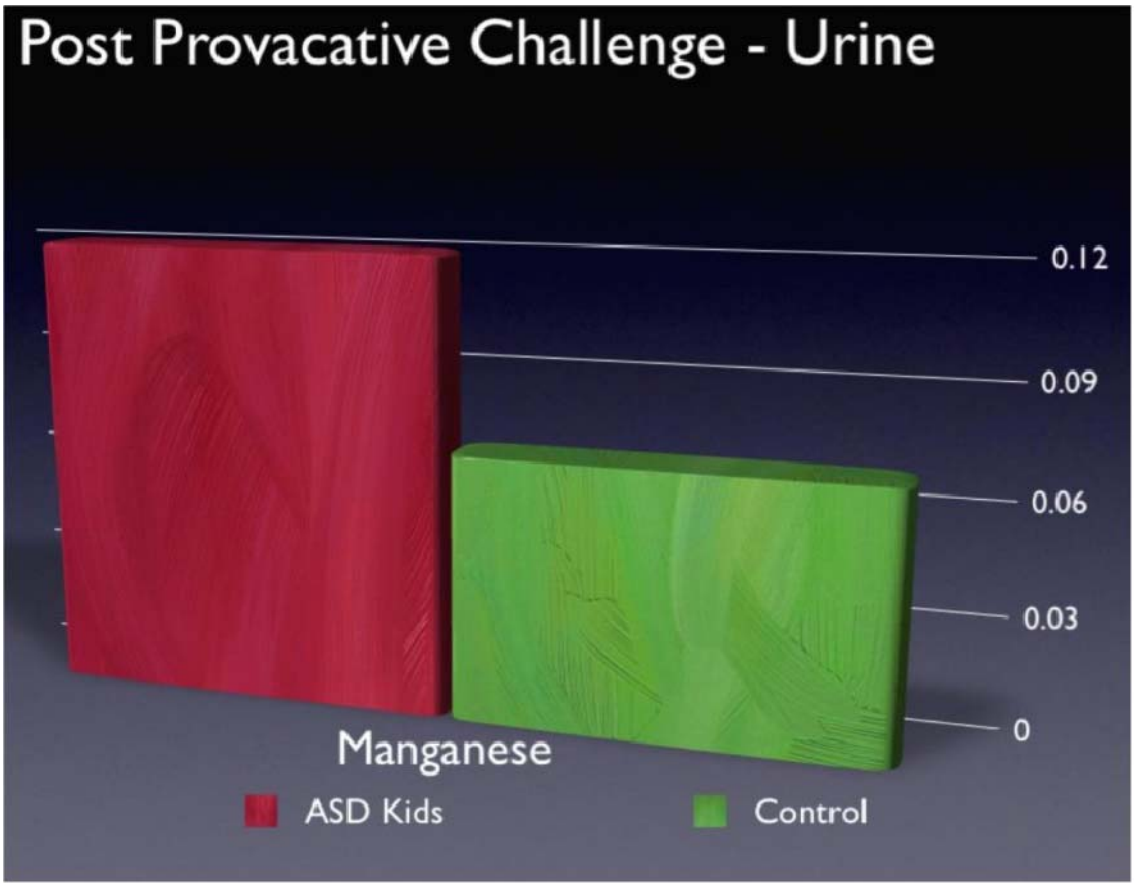
Lead is a neurotoxin to which the developing brain is particularly vulnerable. Moreover, lead poisoning in children is known to negatively affect brain systems implicated in cognitive, communication, and social functioning. [xxxiii] One of the most common causes of neurodevelopmental impairment is childhood lead poisoning – even very low levels.[xxxiv] Pediatric lead poisoning has deleterious effects on the development of widespread brain areas including those implicated in cognitive, communication, and social functioning. In several cases, a temporal association was noted between elevated blood lead levels and the emergence of autistic symptoms. [xxxv]

C. Manganese

a. The amount of manganese was measured pre and post a provocative IV challenge. The 24 hour urine Post

collection for ASD children found manganese levels to be highly elevated as compared to controls.

POST Challenge - Mn
Child ASD mean: 0.12 ug/g creatinine (nl = <0.05) (n=1905)
Controls mean: 0.03 ug/g (n=908)



Manganese

Recent evidence suggests that low-level environmental exposure to manganese adversely affects child growth and neurodevelopment.[xxxvi] Inverse associations have been reported between manganese exposure, measured in environmental and biologic samples, and child cognition, memory, behavior, and motor function.[xxxvii] Results from these studies suggest a possible biphasic dose-response relationship between early-life manganese exposure at lower exposure levels and

infant neurodevelopment. The data are consistent with manganese as both an essential nutrient and a toxicant.

At physiologic levels, manganese protects against oxidative injury, but at high levels, manganese itself is an oxidant. Neurotoxicity from manganese overexposure appears to involve oxidative damage to dopaminergic neurons in particular, as well as mitochondrial dysfunction, which limits energy production and increases oxidative stress and superoxide radical formation.

Together with findings of Takser et al,[xxxviii] who reported adverse effects from fetal manganese exposure, these results suggest that exposure during early life may have the strongest neurotoxic effects.

SNPs and ASD

Genetic predisposition to environmental toxicity is consistent with differential representation of alleles of detoxification genes in ASD subjects versus controls. These genes include methylene tetrahydrofolate reductase (MTHFR), reduced folate carrier (RFC), transcobalamin II (TCN2), Cytochrome P450 (CYP family) catechol-Omethyltransferase (COMT), and glutathione sulfotransferase GST-M. Skew in allele frequencies for paraoxonase (an organophosphate detoxifying enzyme, (PON1) and glyoxalase I (GLO1) in ASD is suggestive of chemical exposure, while bias of ferroportin (SLC40A1/SLC11A3) and metal-activated transcription factor (MTF1) alleles is consistent with heavy metal involvement. [xxxix]
Diminished supply of sulfur-containing amino acids in ASD may also affect susceptibility to environmental agents because these amino acids are critical for some detoxification reactions. Plasma methionine and cysteine were reduced by 39% and 20% respectively in ASD[24]; these are principally derived from the diet and gastrointestinal abnormalities (common in ASD) could be a contributing factor.

ASD is typically diagnosed between two and four years of age, but retrospective analysis has revealed behavioral anomalies from earliest time-points. It seems likely; therefore, that prenatal or perinatal insult is pivotal, concurring with other strong evidence of major risk factors timed during gestation and the perinatal period. Early toxic exposure appears to be critical.

Deficits in detoxification may explain why only a proportion of children develop ASD. There is evidence of a heavy metal mobilization deficiency in affected children. Where susceptibility genes affect detoxification pathways the genetic factor may be in the mother as well as in the child.

Impaired Detoxification

Phase I Detoxification: The First Line of Defense

In Phase I detoxification, enzymes, known collectively as the cytochrome P-450 system, use oxygen to modify toxic compounds, drugs, or steroid hormones. Many toxins must undergo Phase II detoxification after a reactive site has been formed. Because there are many different toxic compounds the body might encounter, there are many variants of Phase I enzymes. These genes include:

- CYP1A1 • CYP2A6 • CYP2E1 • CYP1C19
- CYP1B1 • CYP2D6 • CYP2C9 • CYP3A4

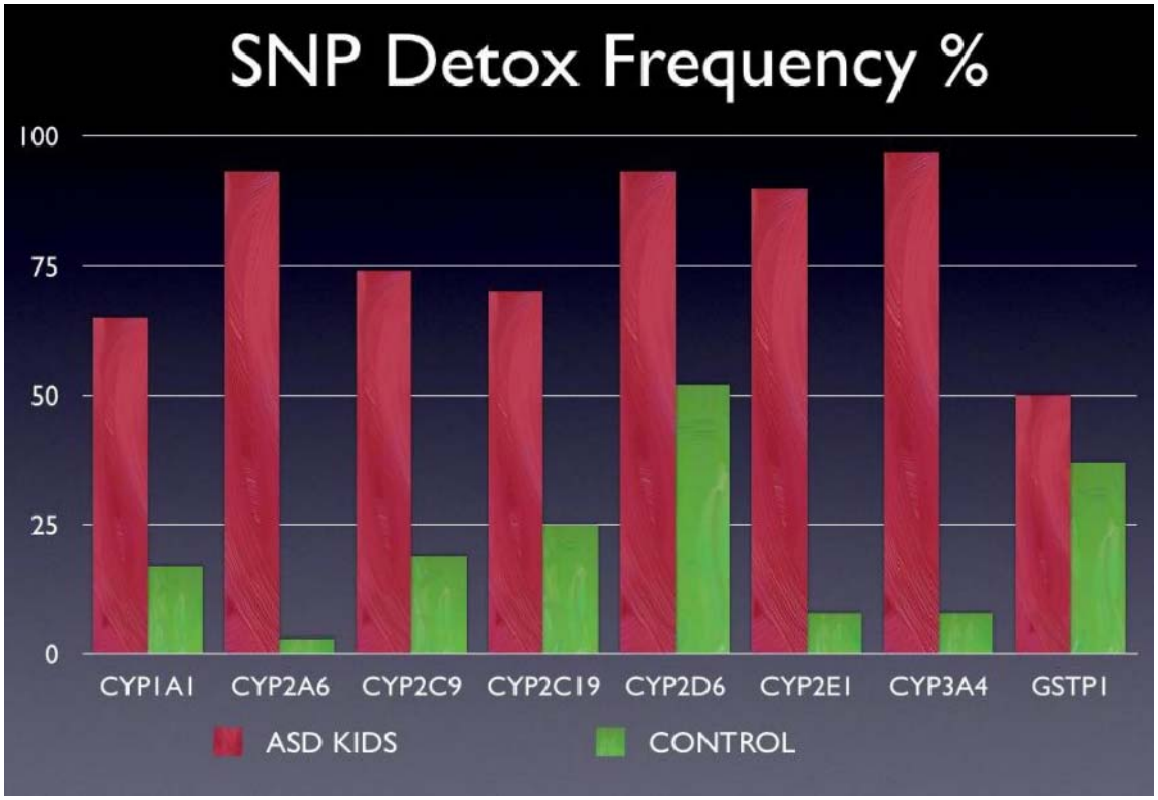
Phase I is the first line of defense in the detoxification of all environmental toxins, including pesticides, herbicides, pollutants, and solvents, pharmaceuticals and nutraceuticals, as well as many of the body's own waste products (including steroid hormones).

Phase II: Conjugation of Toxins

Glutathione Conjugation (glutathione s-transferase)
• GSTM1 • GSTT1 • GSTP1 GST detoxifies many water-soluble environmental toxins, including solvents, herbicides, fungicides, and heavy metals (eg, mercury, cadmium, and lead). Defects in GST activity can contribute to fatigue syndromes and many cancers.

CARE Clinics Detoxification SNP testing data showed polymorphisms in ASD children for the important Phase I and Phase II detox pathways. These results from the CARE Clinic database importantly confirm that children with ASD have compromised detoxification systems and cannot properly breakdown and/or eliminate environmental toxins they have been exposed to.

SNP	ASD (n=358)		Ref %
	#	%	
CYP1A1			
-	125	35	83
+	233	65	17
CYP2A6			
-	27	7	97
+	331	93	3
CYP2C9			
-	94	26	81
+	264	74	19
CYP2C19			
-	106	30	75
+	252	70	25
CYP2D6			
-	11	3	48
+	347	93	52
CYP2E1			
-	34	10	92
+	324	90	8
CYP3A4			
-	48	13	92
+	310	97	8
GSTP1-I105V			
--	137	38	51
-+	179	50	37
++	41	12	12



Impaired Immune System

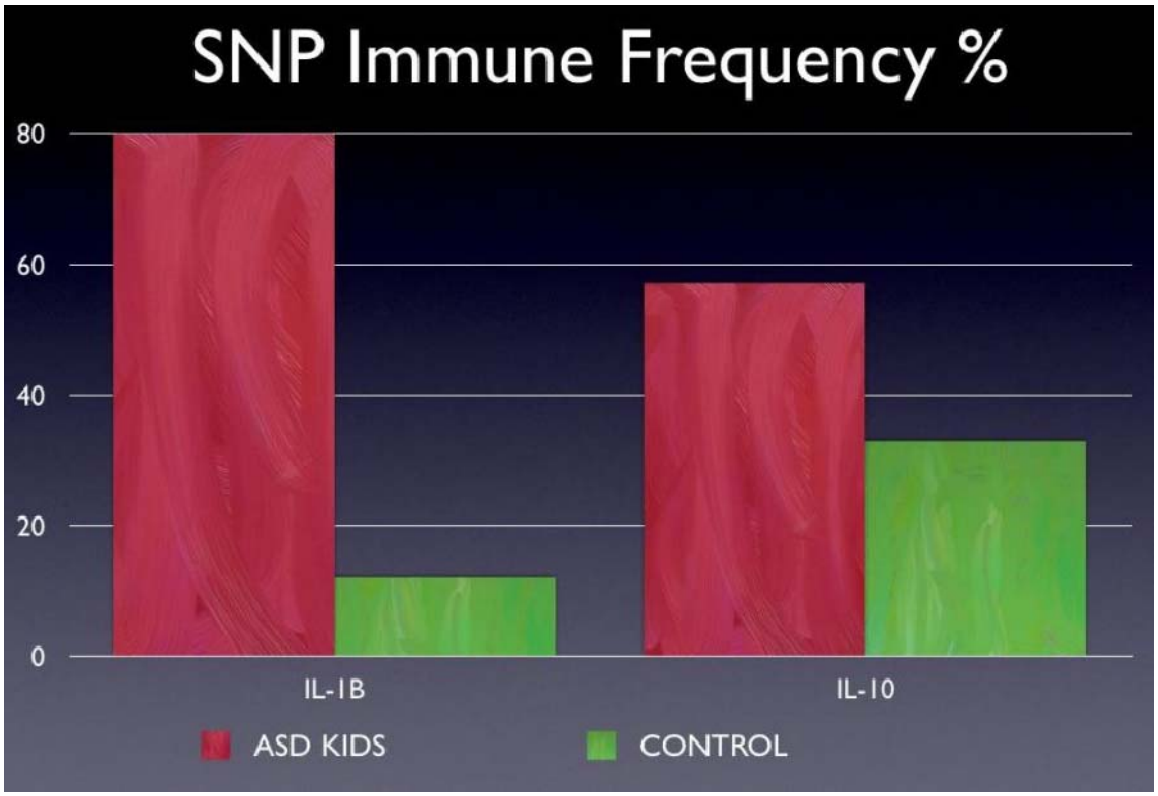
Recent studies have found strong evidence that certain immune system components that promote inflammation are consistently activated in people with autism. [xl] "These findings reinforce the theory that immune activation in the brain is involved in autism" the study authors concluded.

Interleukin 1-beta (IL-1B), produced mainly by blood monocytes, mediates the panoply of host inflammatory reactions collectively known as acute phase response. Polymorphisms in IL-1B may predispose individuals to chronic inflammatory conditions by upregulating COX2 activity and prostaglandin production.

Interleukin – 10 (IL-10) is involved in humoral immunity including the synthesis of IgE and TH-2 cytokines. The measured polymorphisms can result in chronic inflammatory conditions characterized by a TH-1 response.

CARE Clinics data showed significant polymorphisms.

SNP	ASD (n=358)	Ref %
	# %	
IL-1B		
--	81 20	48
-+	163 42	40
++	136 38	12
IL-10		
--	156 43	67
-+	159 45	31
++	43 12	2



Numerous reports have described imbalances in immune and inflammatory processes in autistic patients, including aberrations in

antibody levels, cytokines, and cellular subsets. [xli] [xlii] [xliii] Additionally, recent reports have described an increased frequency of HLA-A2 and HLA-DR4 14 antigens in autism. Interestingly, epidemiological studies have provided evidence for the association of asthma and allergies 15 or autoimmune disorders in families with autistic children [xliv]

Large NIH- and EPA-funded studies are teasing out immune abnormalities that may contribute to autism. In research on more than 700 families with an autistic as well as a neurotypical child, Pessah and his colleagues have found in the autistic child a significant reduction in immunoglobulins and an abnormal profile of cytokines, which are critical to immune response. "The immune system is involved in important aspects of neurodevelopment," says Pessah. "We've found the presence of immune antibodies that we think may influence brain proteins." We hope to find out what type of skewed immune response the typical autistic child has and to isolate toxic exposures, such as proximity to highways or toxic waste dumps."

Herbert argues that "we can address the disturbed pathways now, before the gene hunters have definitive information. Genes, after all, don't specify behaviors. They make regulatory factors that interact in highly complex ways. And as far as the impact of chemicals on neurodevelopment, only about 20 to 30 of the 85,000 chemicals made have been studied. We can, at the very least, try to modulate autism by treating the tissue inflammation."

Gastro-Intestinal Abnormalities

Autistic children often have severe gastro-intestinal symptoms. Such symptoms may be due to a disruption of the indigenous gut flora promoting the overgrowth of potentially pathogenic microorganisms, impairing digestive and absorptive ability. This may be due to decreased output of stomach acid, insufficient production of digestive enzymes and bile and insufficient production of secretin (which stimulates the pancreas to neutralize the stomach acid and to secrete digestive enzymes) and other hormones like CCK (which stimulates the gall bladder to release bile) and Gastric Inhibitory Peptide, which slows the release of acid into the digestive tract.

These digestive concerns promote the overgrowth of yeasts and other potentially harmful microorganisms in the gut. The normal, protective and beneficial microflora (like Lactobacilli and Bifidobacteria) are often found in insufficient numbers. This often causes a drop in the levels of vitamin K, a fat-soluble vitamin that is produced in the intestinal tract by the action of beneficial bacteria on leafy green foods.

CARE Clinics measured the amount of beneficial bacteria in the stool of their ASD patients. Abnormalities were found that are consistent with previously published research. Low levels of the friendly probiotic bacteria – Lactobacillus and Bifidobacter – were found.

Bacteria	ASD Children (n=281) % Abnormal	Controls (n=203) % Abnormal
Lactobacillus	77%	36%
Bifidobacter	48%	13%

This derangement of beneficial flora can lead to dysbiosis – which is a condition of overgrowth of unfriendly gut bacteria. If this goes unchecked long enough, a pervasive and chronic imbalance between colonies will set in, which ultimately minimizes the beneficial nature of these colonies as a whole.

In small amounts the microbial colonies found on or in the body are benign or beneficial in most cases. These beneficial and appropriately sized microbial colonies carry out a series of helpful and necessary functions. They also protect the body from the penetration of pathogenic microbes. These beneficial microbial colonies also compete with each other keeping one another in check so no specific microbial colony dominates.

Microbial colonies also excrete many different types of waste byproducts. Using different waste removal mechanisms, under normal circumstances the body effectively manages these byproducts with little or no trouble. Unfortunately though, over-sized and inappropriately

large colonies, due to their increased numbers, excrete increased amounts of these byproducts. As the amount of microbial byproducts increases, the higher waste byproducts levels can overburden the body's waste removal mechanisms.

A new study conducted by Autism Speaks' Autism Treatment Network (ATN) shows that GI symptoms occur in nearly half of children with ASD, and the prevalence increases as children get older. Data from 1,185 children showed that 45 percent had GI symptoms at the time of enrollment, with abdominal pain, constipation and diarrhea reported most commonly. Results of the study, were presented on May 2 at the Pediatric Academic Societies (PAS) annual meeting in Vancouver, British Columbia, Canada.

It has been observed that children with autism have malabsorption in addition to the gut flora dysbiosis.[xlv] Additionally malabsorption has been implicated in the cause of cerebral dysfunction in autistic children. [xlvi] Dysbiosis can lead to the overgrowth of harmful bacteria such as Clostridia spp. which has been found to produce HPHA (a tyrosine analog) which depresses brain catecholamines and cases symptoms of autism. [xlvii]
Inflammation of the entire alimentary canal is common in autism with some researchers finding esophagitis and duodenitis in 70%. [xlviii] The inflamed gut leads to malabsorption and/or leaky gut. Intestinal Oxidative stress is aggravated by microbial overgrowth, exogenous and endogenous toxins not being processed efficiently and exposure to toxic metals.

Malabsorption

Additional laboratory findings at CARE Clinics showed further indications of malabsopbtion and dysbiosis through the measurement of Organic Acids and Plasma Amino Acids.

In ASD children levels of Arabinose (breakdown of hyluronic acid), Indoleacectic Acid (produced from breakdown of unabsorbed tryptophan) and 5-OH-indoleacectic acid (5-HIAA) were elevated above the reference ranges. These organic acids are typically the result of

malabsorption, gut bacterial action and in some cases hepatic detoxification of chemicals produced by dysbiotic flora. 5-HIAA is a marker for increased levels and release of serotonin from the gut (possible diarrhea predominant IBS). ASD n=164. Control n=54.

Arabinose – 48% of ASD children had levels above reference range while the controls had 16% above reference range.
Indoleacectic Acid – 38% of ASD children had levels above reference range while the controls had 7% above range.
5-HIAA – 42% of ASD children had levels above reference range while the controls had 6% above reference range.

Allergies

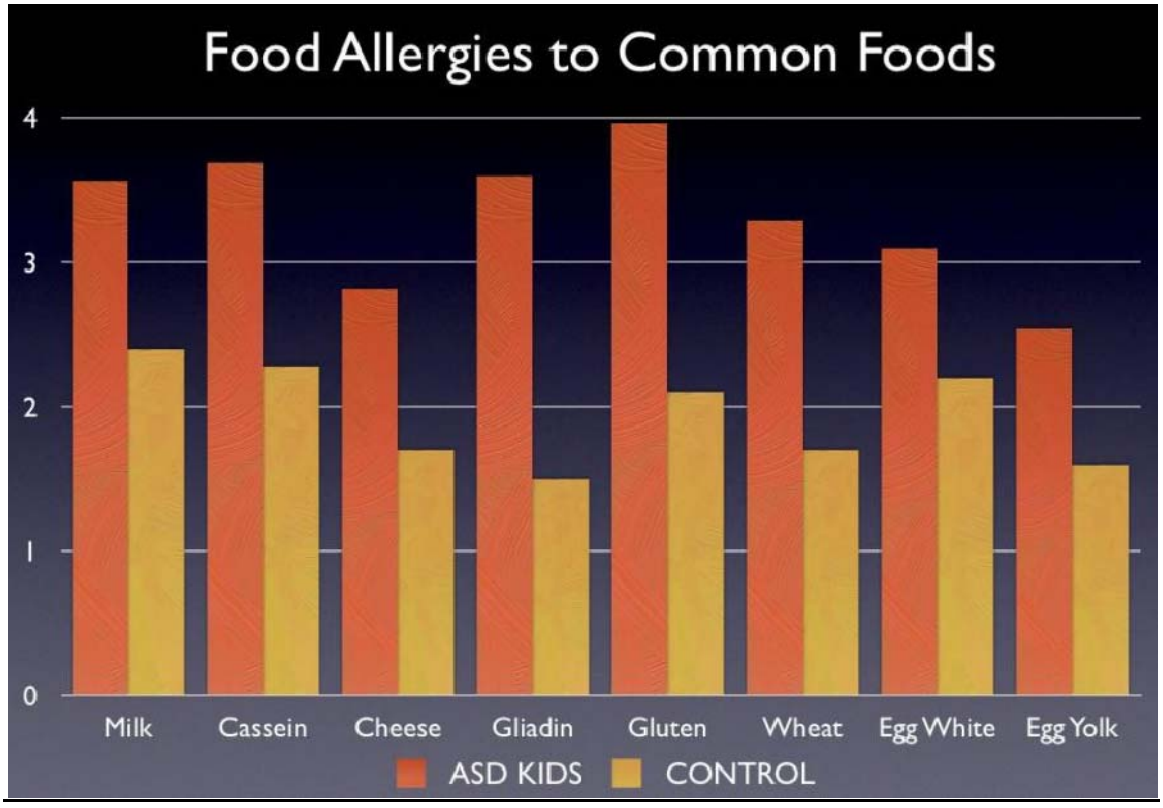
It's been suggested that autism could result from a loss of regulation of the immune system, causing an increase in inflammatory-causing chemical signals from white blood cells. [xlix]It is felt that these chemicals (cytokines) may be responsible for the neurological abnormalities seen in children with autism.

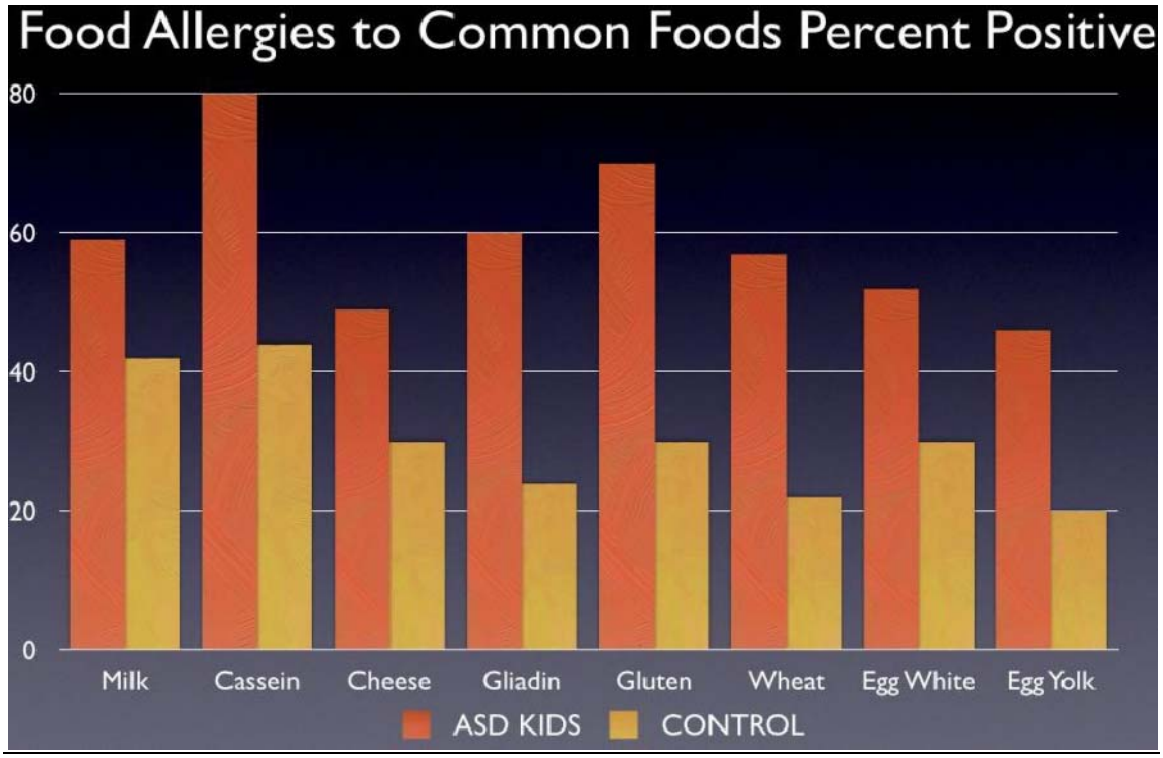
Recent studies suggest that children with autism may respond to certain foods, particularly gluten- and casein-containing foods, by producing more of these inflammatory cytokines. Blood cells from autistic children were cultured with various foods in a lab, and various inflammatory cytokines were measured. The cytokines from the autistic children were much higher than those from normal (non-autistic) children after being exposed to gluten or casein. Mothers of autistic children were more likely to have asthma or allergic disorders than the mothers of healthy control children in a study of 2,500 children, said Lisa Croen, Ph.D., a researcher at Kaiser Permanente Northern California, Oakland. Additional research suggests that certain food peptides might be causing toxic effects at the central nervous system by interacting with neurotransmitters. [l]

CARE Clinics Results from IgG (Type 4 delayed hypersensitivity) blood allergy testing at CARE Clinics confirmed that indeed many ASD children were highly allergic to various common foods. IgG allergy effects may

include inflammation, ADD, behavior/personality changes and irritability. Additional effects may include the irritation of the gut wall leading to increased intestinal permeability (leaky gut). The titer score was from 0 to 4. ASD n=251 while the controls n=1544.

	ASD		Control	
	Mean	% Positive	Mean	% Positive
Milk	3.56	58%	2.14	48%
Cassein	3.69	80%	2.02	50%
Cheese	2.81	49%	2.00	33%
Gliadin	3.60	61%	1.80	28%
Wheat Gluten	3.96	70%	2.18	33%
Wheat	3.29	57%	1.70	22%
Egg White	3.10	53%	1.2	31%
Egg Yolk	2.54	46%	1.92	22%





Infections

Herpes Connection

Certain cases of autism have been reported to be caused by disorders that produce structural abnormalities in the brain or space occupying lesions as well as by brain infections. Herpes encephalopathy can produce all of the core symptoms of autism and, similar to the second patient, appear well after the age typically associated with the onset of idiopathic autism. [li] Indeed, there are case reports of herpes-induced autism in previously healthy adolescents and in an adult of 31 years. [lii]

CARE Clinics measured HHV-6 IgG Antibodies and the results showed 48% of the ASD children (n=209) positive versus 7% of the controls (n=84)

The Streptococcal Connection

Several specific environmental exposures have been found to confer a greatly increased risk for autism. An epidemic of rubella in the late 1960s revealed that congenital exposure was associated with dramatically elevated incidence rates, as high as 1 in 25 exposed children, far higher than prevailing rates in the population during that same time period. Further research in humans and in experimental animals has supported a possible role for maternal viral infections. These microbes may act on the developing central nervous system The streptococcus germ (usually referred to as the "strep" organism) is well known for causing infections like sore throats and impetigo. Chronic strep carriage is not uncommon. In some populations as many as 25% of the people will harbor strep in their throats. Some individuals experience autoimmune reactions after a strep infection that can damage the heart (rheumatic fever), kidneys (glomerulonephritis) or the brain (Chorea, OCD or PANDAS).

Chorea refers to the strange and inappropriate movements that some individuals experience after strep infections. OCD is obsessive, compulsive disorder and PANDAS is an acronym for "pediatric autoimmune psychiatric disorders associated with streptococcus", and all these disorders have been observed in certain susceptible children after a strep infection.

PANDAS can manifest as peculiar behaviors or motor disturbances. Dr. Vojdani of Immuno Sciences Lab has found antibodies to the strep M protein as well as autoantibodies against neuronal tissue in samples taken from autistic children. [liii] Strep infection can promote the production of certain inflammatory substances like tumor necrosis factor (TNF) and nuclear factor kappa B (NFK-B). High levels of TNF are seen in those with tic disorders and in those with OCD.

Strep germs produce a number of troubling substances. These include streptokinase, which can increase TNF and IL6, another inflammatory mediator, and NADase, an enzyme that depletes NAD, which is necessary for recycling glutathione. TNF and IL6 are known to decrease methylation, which would serve to aggravate the 85% of autistic

children who are undermethylated to begin with, and methylation reactions are necessary for the proper myelination of nerves and the "pruning" of excessive brain neurons. Autistic children show myelination delays in the outer area of white matter of the brain consistent with this hypothesis.

A recent study showed that late onset autism was a consequence of probable autoimmune processes related to chronic bacterial infection. [liv] CARE Clinic patient database showed elevated levels of Anti-Strep antibodies.

The mean for Anti-Strep Dnase B (nl = <200 U/ml)

ASD Kids: 267 U/ml (n=236)
Controls: 134 U/ml (n=145)

above ref range:
ASD kids: 62%
Controls: 14%

The mean for Anti-Streptolysin 0 (nl =<200 U/ml)

ASD Kids: 228 U/ml (n=236)
Controls: 122 U/ml (n=145)

above re range:
ASD Kids: 49%
Controls: 11%

Uric Acid

Low levels of uric acid can inhibit axonal growth in the brain and are an indicator of increased oxidative stress. Oxidative stress is linked to the clinical symptoms and pathogenesis of autism through linking with membrane lipid abnormalities, inflammation, aberrant immune response, impaired energy metabolism and excitotoxicity. [lv] Low levels

are also associated with lead toxicity, folic acid deficiency, autistic symptoms and immune deficiency.

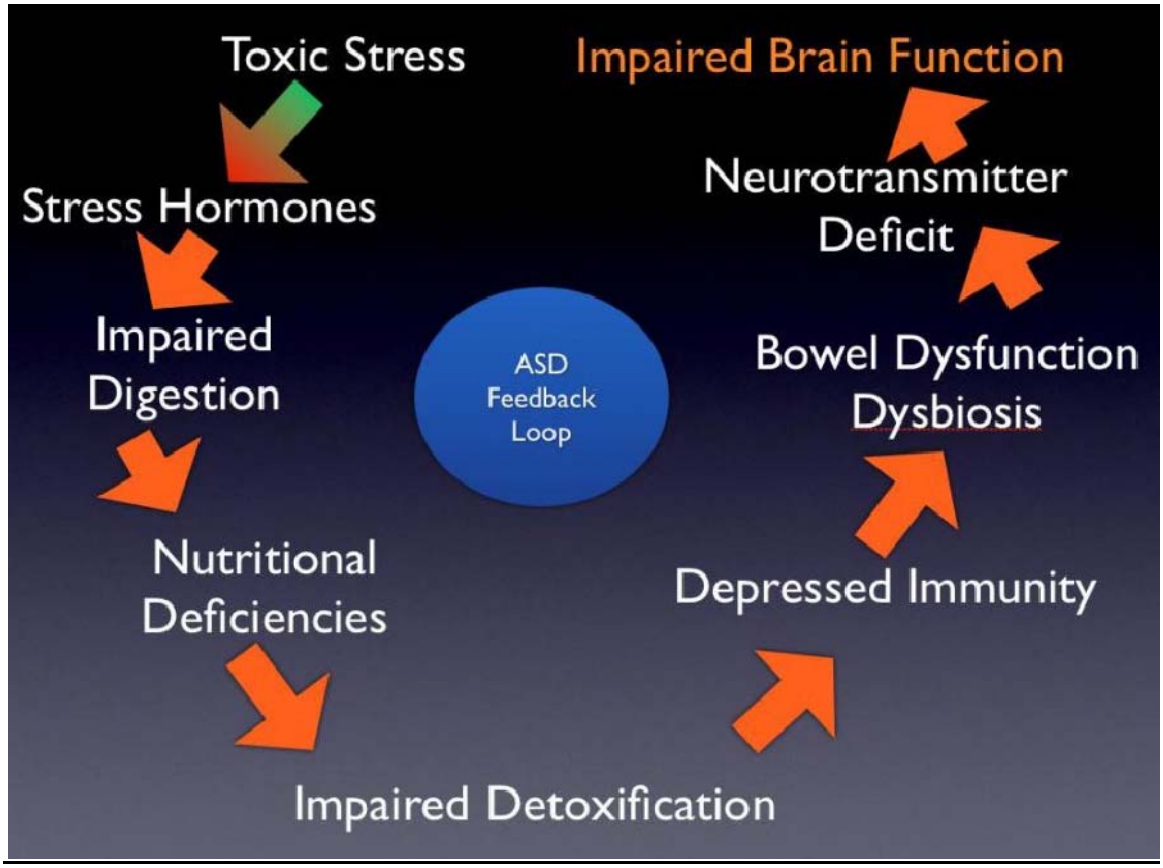
CARE Clinics ASD patients showed low levels of uric acid in their urine (Uric Acid/ Creatinine ratio).

> ASD children (n=114) had a mean of .45 as compared to ref range of >6 with 75% of the children having low levels.

Conclusion

The CARE Clinics ASD clinical database analysis clearly supports previously proposed and researched genetic predispositions of ASD children along with the concomitant abnormalities in various physiological parameters that postulate various etiological causes of ASD and points in the direction of both appropriate treatment and interventions to avoid the triggering of ASD.

The analysis also supports the possible mutifactorial cause of ASD (as shown in the following chart) and the appropriateness of individualized treatment based on laboratory findings.



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Author, medical researcher, entrepreneur

Dr. Barrie was in clinical practice during the mid 1980's at the Great Smokies Medical Center, specializing in integrative medicine and clinical ecology. He was the founder and former CEO/Chairman of Great Smokies Diagnostic Laboratory (now Genova Diagnostics), an international clinical reference laboratory for physicians, which he started in 1986 to advance natural medicine and the scientific research of integrative medicine around the world. Dr. Barrie developed many of the present day laboratory tests used for understanding the root causes of many chronic diseases. Dr. Barrie is considered an expert in the field of personalized medicine – identifying an individual's susceptibility to disease; how a person will respond to a particular treatment; and what is the best treatment.

He has been a pioneer in promoting the concepts of preventive medicine and early intervention as the most rational and cost effective healthcare model, and has been a speaker in numerous worldwide forums presenting the scientific basis for Functional Medicine and sharing new ways to uncover the causes of common diseases. Dr. Barrie is also a NY Times and LA Times best-selling author of several health books including "7 Day Detox Miracle", "Energize Your Life" and numerous scientific articles, which have appeared in major medical journals including *The Lancet*, *Agents and Actions* and *Medical Hypothesis.*

Dr. Barrie also pioneered the concept of "home" and "direct to consumer" laboratory testing and developed the first commercially viable SNP Genomic testing profiles for common diseases; was co-founder of iNutritionals, the country's leading developer and provider of supplements and tools for brain health.